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(54) TYROSINASE-ACTIVITY INHIBITOR AND BEAUTIFYING COSMETICS USING THE SAME

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain a tyrosinase-activity inhibitor that develops excellent tyrosinase-activity inhibitory action, and is useful as a beautifying cosmetic material by the use of a carboxylic acid of a specific structure which is obtained from royal jelly.

SOLUTION: This tyrosinase-activity inhibitor comprises one or more carboxylic acids selected from a group of C8 or C10 carboxylic acids consisting of o-hydroxydecanic acid, decanic acid, 2-decanoic acid, 3-hydroxydecanoic acid, sebasic acid, trans-2-octenoic acid, octanoic acid, 3-hydroxyoctanoic acid and suberic acid. These carboxylic acids are preferably royal jelly-derived compounds obtained from royal jelly by extraction. The extract from royal jelly has no skin irritation, and has excellent adaptability to skin. It is preferable to add approximately 1-20wt.% of this tyroxynase-activity inhibitor to a beautifying cosmetic.

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**CLAIMS**

[Claim(s)]

[Claim 1] 10-hydroxydecanoic acid, decanoic acid, 2-decanoic acid, 3-hydroxydecanoic acid, Tyrosinase activity inhibitor in which a carbon number which consists of sebamic acid, transformer 2-octenate, octanoic acid, 3-hydroxyoctanoic acid, and suberic acid consists of one or more sorts of carboxylic acid chosen from the carboxylic acid of 8 or 10.

[Claim 2] The tyrosinase activity inhibitor according to claim 1 which is a compound of royal jelly origin of said carboxylic acid.

[Claim 3] Skin-whitening cosmetics containing the tyrosinase activity inhibitor according to claim 1 or 2.

[Translation done.]

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## DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to tyrosinase activity inhibitor for controlling generation of melanin, and the skin-whitening cosmetics using it.

[0002]

[Description of the Prior Art] Melanin is coloring matter which has determined the color of hair or skin. In the melanocyte which exists in the stratum basale epidermidis of the skin, this coloring matter is generated in response to a superfluous light and ultraviolet rays. the generation process of melanin uses tyrosine as a precursor -- tyrosine  $\rightarrow$  L-DOPA  $\rightarrow$  dopa quinone  $\rightarrow$  -- dopa -- pass the process of chromium  $\rightarrow$  5,6-dihydroxyindole  $\rightarrow$  indole- 5,6-quinone  $\rightarrow$  melanin -- it is generated.

[0003] In this process, a tyrosinase involves in process of a tyrosine  $\rightarrow$  L-DOPA  $\rightarrow$  dopa quinone, and the following processes are made by autoxidation. Therefore, when searching a melanin generation inhibitor, the method of seeing the activity inhibition of a tyrosinase has been taken. It is an important factor of whitening effect to have tyrosinase activity inhibitory action.

It is the controlling method for having made the main force of skin-whitening cosmetics since here about ten years.

[0004] Arbutin, kojic acid, ascorbic acid, etc. are one of those based on tyrosinase activity inhibitory action are typical as a melanin generation inhibitor, it applies also for many cross references to related application to each compound, and the cosmetics which actually blended these are produced commercially. Although there is a report (JP,2-193917,B) of Mishima and others about the melanin generation depressant action in which carboxylic acid participates, The carboxylic acid shown there is not only a thing about the unsaturated fatty acid of the

carbon numbers 12 thru/or 22, acetic acid, lactic acid, and pyruvic acid, but the mechanism of action controls generation of a tyrosinase. Tyrosinase activity inhibitor mainly expects the whitening effect now, and research and development are made.

[0005]

[Problem(s) to be Solved by the Invention]However, although said ascorbic acid checks tyrosinase activity by the reducing action, it cannot say the operation with sufficient thing. The effect is insufficient although arbutin also has tyrosinase activity inhibitory action. Although tyrosinase activity inhibitory action is also excellent, it was isolated from the culture medium of the Aspergillus which gives a color and flavor to bean paste, soy sauce, etc., and kojic acid is not only a natural product, but when iron exists, there is a possibility that it may become blackish brown and quality may deteriorate. For this reason, that quality was good in the substance of natural product origin, and development of what has the outstanding tyrosinase activity inhibitory action was desired.

[0006]This invention is made paying attention to the problem which exists in the above conventional technologies. There is a place made into the purpose in providing tyrosinase activity inhibitor excellent in tyrosinase activity inhibitory action. The place made into the other purposes has a possibility of discoloring in providing tyrosinase activity inhibitor with little sufficient quality, and the skin-whitening cosmetics using it while there is no skin irritation and having a desire for nature.

[0007]

[Means for Solving the Problem]In order to attain the above-mentioned purpose, tyrosinase activity inhibitor of the invention according to claim 1, 10-hydroxydecanoic acid, decanoic acid, 2-decenoic acid, 3-hydroxydecanoic acid, A carbon number which consists of sebacic acid, transformer 2-octenate, octanoic acid, 3-hydroxyoctanoic acid, and suberic acid consists of one or more sorts of carboxylic acid chosen from the carboxylic acid of 8 or 10.

[0008]Tyrosinase activity inhibitor of the invention according to claim 2 consists of a compound of royal jelly origin of said carboxylic acid. Skin-whitening cosmetics of the invention according to claim 3 contain claim 1 and the tyrosinase activity inhibitor according to claim 2.

[0009]Therefore, the tyrosinase activity inhibitor according to claim 1 consists of specific carboxylic acid, and reveals outstanding tyrosinase activity inhibitory action, and carboxylic acid checks the activity of a tyrosinase in a process which a dopa quinone generates from tyrosine. As a result, generation of melanin is controlled.

[0010]Although the tyrosinase activity inhibitor according to claim 2 is carboxylic acid of royal jelly origin, it has the outstanding tyrosinase activity inhibitory action from containing said specific carboxylic acid. And royal jelly is widely used also as drugs or health food, and a compound of royal jelly origin does not have skin irritation, and agrees in a desire for nature.

[0011]The skin-whitening cosmetics according to claim 3 contain the tyrosinase activity

inhibitor according to claim 1 or 2, it is well absorbed on the skin, an activity inhibition operation of a tyrosinase is revealed, and whitening effect is obtained.

[0012]

[Embodiment of the Invention] Below, the embodiment of this invention is explained in full detail. Tyrosinase activity inhibitor 10-hydroxydecanoic acid, decanoic acid, The carbon number which consists of 2-decenoic acid, 3-hydroxydecanoic acid, sebacic acid, transformer 2-octenate, octanoic acid, 3-hydroxyoctanoic acid, and suberic acid contains one or more sorts of carboxylic acid chosen from the carboxylic acid of 8 or 10. These carboxylic acid has a double bond, a carboxyl group, or hydroxyl, according to the purpose, one of kinds of they can be chosen, and it can use, or it can choose two or more sorts suitably, and they can be used for it.

[0013] Since it is a thing of the desire for nature obtained from a natural product that it is a compound of the royal jelly origin produced by extracting from royal jelly, these carboxylic acid is desirable. That is, the extract from royal jelly does not have skin irritation, and is excellent in the applicability to skin. Since these tyrosinase activity inhibitor can control generation of melanin for the activity of a tyrosinase by inhibition \*\*\*\*\*, it can demonstrate whitening effect.

[0014] Here, how to extract carboxylic acid from royal jelly is explained. First, alcohol is added to raw royal jelly and mobility is received. Subsequently, vacuum concentration of the filtrate produced by carrying out processing according this to an ultrafiltration method or a precision filtration process is carried out, and carboxylic acid is obtained as a crystal by cooling. An ultrafiltration method is a method of filtering particles using the ultrafiltration membrane which consists of a porous poly membrane which has an aperture of  $1 - 10^3$  nm, and a precision filtration process is a method of filtering particles using the micro filter which consists of a porous poly membrane which has a 0.02-10-micrometer aperture.

[0015] Next, skin-whitening cosmetics are obtained by making a makeup substrate contain the above tyrosinase activity inhibitor. A milky lotion, face toilet, cream, etc. are used as a makeup substrate. The content of tyrosinase activity inhibitor has 1 to 20% of the weight of a preferred range. At less than 1 % of the weight, even if the operation which checks the activity of a tyrosinase is low, and sufficient whitening effect is not obtained but it exceeds 20 % of the weight, such an effect does not improve but cost goes up on the contrary.

[0016] The shape of a milky lotion, and any creamy and powdered any may the gestalten of skin-whitening cosmetics be? By applying such skin-whitening cosmetics to skin, tyrosinase activity inhibitor in skin-whitening cosmetics is absorbed by skin, and controls the melanin generated on the surface of skin. Therefore, skin can be kept fair.

[0017] The activity of a tyrosinase uses L-DOPA as a substrate, measures the quantity of the DOPA chrome generated as a result of an enzyme reaction, and is calculated from the molar extinction coefficient. That is, L-DOPA is added and after making a tyrosinase and tyrosinase

inhibitor add and react to a phosphate buffer solution, is made to react. Then, acetic acid is added 50%, a reaction is stopped, and the absorbance at 475 nm is measured. And the grade of tyrosinase activity inhibition can be calculated from the decrement of the absorbance obtained by adding tyrosinase inhibitor.

[0018]The check of whitening effect gets the panel test which added tyrosinase activity inhibitor to common cosmetic cream, i.e., two or more panelists, to use cosmetics, and is performed based on the decision result.

[0019]Thus, according to tyrosinase activity inhibitor and skin-whitening cosmetics which are obtained, the following effects are demonstrated.

(1) 10-hydroxydecanoic acid, decanoic acid, 2-decenoic acid, 3-hydroxydecanoic acid, As for the specific carboxylic acid of 8 or 10, the carbon number which consists of sebacic acid, transformer 2-octenate, octanoic acid, 3-hydroxyoctanoic acid, and suberic acid is a long chain aliphatic compound, and has a double bond, a carboxyl group, hydroxyl, etc. Based on the specific structure of such carboxylic acid, tyrosinase activity inhibitor is well absorbed on the skin, and the outstanding tyrosinase activity inhibitory action is demonstrated.

(2) Many carboxylic acid of 8 or 10 is contained in the compound extracted from royal jelly, and a carbon number has the outstanding tyrosinase activity inhibitory action to it.

(3) Royal jelly is a natural product which a worker bee produces, the compound extracted from the royal jelly agrees in a desire for nature, and there is little stimulativeness over the skin.

(4) The carboxylic acid of royal jelly origin is based on the production thing of a bee as well as honey, it does not have discoloration compared with the kojic acid based on bean paste or soy sauce, and its quality is high.

(5) Since said carboxylic acid is excellent in tyrosinase activity inhibitory action, it can control generation of melanin effectively and can demonstrate the whitening effect outstanding based on it.

[0020]

[Example]

(Example 1) After adding 3 kg of ethanol to 1 kg of raw royal jelly 95% of the weight and dissolving in it, diatomaceous earth filtration was carried out, precision filtration was further carried out using the micro filter with the aperture of 5 micrometers or less, and 3.42 kg of filtrate was obtained. Vacuum concentration of this was carried out and the crystal of a 63-g royal jelly extract was obtained. During this crystal, the carboxylic acid which makes the start 30% of the weight of 10-hydroxydecanoic acid is contained.

[0021]The percentage of the carboxylic acid contained in royal jelly is shown in the following table 1. Thus, the carbon number obtained the tyrosinase activity inhibitor which consists of carboxylic acid of 8 or 10.

[0022]

[Table 1]

成 分	構成比(重量%)
1,0-ヒドロキシデセン酸	3.0
グルコン酸	2.4
1,0-ヒドロキシデカン酸	2.2
8-ヒドロキシオクタン酸	4
p-ヒドロキシ安息香酸	3
3-ヒドロキシデカン酸	2
3,10-ヒドロキシデカン酸	2
その他	1.3

[0023]The carbon number shown in Table 2 prepared the tyrosinase activity inhibitor which consists of carboxylic acid of 8 or 10 as (Examples 2-10 and the comparative example 1), next Examples 2-10.

[0024]The tyrosinase activity inhibitor which consists of conventional arbutin as the comparative example 1 was prepared.

(Example 1 of an examination) By the examining method shown below, the tyrosinase activity inhibitory action was checked about each sample of arbutin, the above-mentioned Examples 1-10, and the comparative example 1. That is, after adding 0.1 ml of tyrosinase solutions, and 0.1 ml of sample solutions to 2.7 ml of 0.104M phosphate buffers (pH 6.0) and incubating for 5 minutes at 25 \*\*, 0.1 ml of 30mM L-DOPA solutions were added, and the reaction was performed for 15 minutes at 25 \*\*.

[0025]Next, 0.26 ml of acetic acid was added 50%, the enzyme reaction was stopped, the absorbance at 475 nm was measured, and it asked for the sample concentration which checks tyrosinase activity 50%. The result is shown in Table 2. Although + in Table 2 prevents, it is shown that  $IC_{50}$  is not calculated, and \*\* shows that inhibition is slight.

[0026]

[Table 2]

実施例 または 比較例	試 料	IC <sub>50</sub> 濃度 (mM)
実施例 1	ローヤルゼリー抽出物	1. 2 0
実施例 2	1,0-ヒドロキシデカン酸	1. 4 0
実施例 3	デカン酸	1. 7 0
実施例 4	2-デセン酸	0. 9 0
実施例 5	3-ヒドロキシデカン酸	±
実施例 6	セバチン酸	2. 1 5
実施例 7	トランス-2-オクテン酸	1. 2 0
実施例 8	オクタン酸	1. 7 0
実施例 9	3-ヒドロキシオクタン酸	±
実施例 10	スペリン酸	3. 1 5
比較例 1	アルブチン	+

[0027]As shown in Table 2, there is a difference of a grade in the carbon number 8 or the carboxylic acid of 10 in Examples 2-10, but there is the tyrosinase activity inhibition effect of having excelled compared with the arbutin of the comparative example 1. The same effect as

the carboxylic acid extracted from the royal jelly of Example 1 is acquired.

(Example 11) The whitening cream which blended the royal jelly extract obtained from Example 1 was manufactured according to the following formulas. It was made for the total quantity to serve as 100 weight sections.

(1) **処方**

油相	スクワラン	2.0 重量部
	オリーブ油	4 重量部
	ローヤルゼリ抽出物	4 重量部
	グリセリンモノステアレート	4 重量部
水相	キサンタンガム	1 重量部
	香料、防腐剤	適量
	精製水	6.5 重量部

(2) Mix uniformly method-of-preparation \*\*\*\* and an oil phase ingredient, and carry out heating churning for 5 minutes at 75 \*\*. Subsequently, the aqueous phase component (75 \*\*) was added to this solution, it emulsified until it became uniform, and creamy skin-whitening cosmetics were obtained.

(Example 2 of an examination) Whitening effect was tested using the base cream which does not blend the cream obtained in Example 11, and royal jelly. Panelists are 20 women, used it continuously for three months, and checked whitening effect. And when a panelist judged that it is effective in the improvement of swarthiness, silverfish, and a freckle, and it was judged that it is a little effective, it classified into three at the time of judging that it is invalid. The result is shown in Table 3.

[0028]

[Table 3]

	効能	実施例 11 のクリーム	基剤 クリーム
美白効果	有効 やや有効 無効	14人 3人 3人	9人 3人 17人

[0029]It was checked that the cream of Example 11 demonstrates swarthiness, silverfish, and the whitening effect excellent in the improvement of a freckle so that clearly from Table 3. In this test, the reaction which is not preferred on the skin was not observed at all.

[0030]The technical idea grasped from said embodiment is indicated below.

(1) The tyrosinase activity inhibitor according to claim 1 which is a compound in which said carboxylic acid has a double bond.

[0031]When constituted in this way, an activity inhibition operation of a tyrosinase can be raised by the interaction of said specific structure of carboxylic acid, and a double bond.

(2) The tyrosinase activity inhibitor according to claim 1 in which said carboxylic acid is one or

more sorts of compounds in which a carbon number is chosen from the carboxylic acid of 10. [0032]When constituted in this way, the interaction of said specific structure of carboxylic acid and the long chain compound which has a predetermined carbon number can raise an activity inhibition operation of a tyrosinase.

(3) The tyrosinase activity inhibitor according to claim 1 which is a compound in which said carboxylic acid has hydroxyl.

[0033]When constituted in this way, an activity inhibition operation of a tyrosinase can be raised by the interaction of the specific structure of carboxylic acid, and hydroxyl.

(4) The tyrosinase activity inhibitor according to claim 2 produced by condensing the filtrate which carried out filtration treatment after said carboxylic acid adds alcohol to raw royal jelly.

[0034]If constituted in this way, the carboxylic acid of royal jelly origin is efficiently acquirable.

(5) Tyrosinase activity inhibitor given in the above (4) which is what depends said filtration treatment on an ultrafiltration method or a precision filtration process.

[0035]When constituted in this way, the carboxylic acid of royal jelly origin can be acquired more efficiently.

[0036]

[Effect of the Invention]Since this invention is constituted as mentioned above, it does the following effects so. According to tyrosinase activity inhibitor of the invention according to claim 1, the tyrosinase activity inhibitory action which was excellent when the carbon number which has specific structure used the carboxylic acid of 8 or 10 can be demonstrated.

[0037]While being able to obtain the whitening effect based on the outstanding tyrosinase activity inhibitory action according to tyrosinase activity inhibitor according to claim 2 and the skin-whitening cosmetics according to claim 3, By using the compound extracted from royal jelly, skin irritation can be controlled, it can agree in a desire for nature, and the quality of being able to control discoloration can be raised.

[Translation done.]